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STUDIES ON THE REPRODUCTIVE SYSTEMS OF SEA-STARS. I. THE MORPHOLOGY AND HISTOLOGY OF THE GONAD OF *ASTERIAS VULGARIS* [1]

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Although the gonads of *Asterias vulgaris* are part of one of the major organ systems in this sea-star they have not been the subject of a basic morphological or histological study other than the preliminary account published by Field in 1892. The structure of the reproductive system has been described with varying degrees of completeness in a wide variety of other sea-stars (*Asterias rubens,* Hoffmann, 1872; *Asterias rubens* and *Asterina gibbosa,* Ludwig, 1877, 1878; *Asterias rubens,* Hamann, 1885; *Asterina gibbosa,* Cuénot, 1887; *Solaster endeca* and *Asterias rubens,* Gemmill, 1911, 1912 and 1914; *Asterina batheri,* Hayashi, 1935; *Coscinasterias tenuispina,* Cognetti and Delavault, 1958; *Echinaster sepositus,* Delavault, 1960; *Marthasterias glacialis,* Delavault and Cognetti, 1961; *Odontaster validus,* Pearse, 1965; *Pisaster ochraceus,* Mauzey, 1966; *Asterina gibbosa* and *Echinaster sepositus,* Tangapregassom and Delavault, 1967; *Leptasterias hexactis,* Chia, 1968; *Asterina gibbosa,* Bruslé, 1969; *Henricia sanguinolenta,* Chia, 1970; and *Astropecten armatus, Henricia leviuscula, Patiria miniata, Pisaster ochraceus,* Davis, 1971), but no comprehensive, detailed, and accurate description has been published for any sea-star which discusses the structure of the gonads based on observations with both the light and electron microscopes.

In the present study, observations have been made on both sexes of *Asterias vulgaris,* involving principally the morphology and histology of the gonads; notes are included on the ultrastructure of the tissues of the gonad and also on modifications occurring in the structure of the gonad during a single annual reproductive cycle. The information provided here should be useful to students of the physiology of reproduction in asteroids and will also provide a basis for comparative studies on the reproductive system in other asteroids.

Materials and Methods

Collection and identification of specimens

All specimens of *Asterias vulgaris* used in this study were collected at the Isles of Shoals, a group of islands in the Gulf of Maine about 10 miles southeast of Portsmouth, New Hampshire. The principal collection site for this work was the leeward face of a breakwater stretching in a north-south direction from Cedar Island to Star Island. A series of four collections of sea-stars (referred to as "major collections") was made at this site in October and December, 1970 and in

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March and July, 1971. Information gathered by Sherman (1966) about the reproductive cycle of *Asterias vulgaris* on the north side of Cape Cod was used as a guide in selecting the dates of these collections. At her study site, *Asterias vulgaris* showed a poorly defined yearly reproductive cycle, including a period of gametogenesis from October until March when gametogenesis was complete. Spawning followed during July and continued until August; the resulting spent gonads were then quiescent in terms of gamete production again until October. The "major collections" were made corresponding to Sherman's data; in this way specimens with gonads demonstrating (1) active gametogenesis, (2) completed gametogenesis, and (3) no gametogenesis (spent gonads) were obtained. Such specimens were used to observe changes in the basic histology of the gonad resulting from the activity of the germinal epithelium.

In order to determine that the species used in this study was indeed *Asterias vulgaris* and not *Asterias forbesi,* the set of criteria outlined by Aldrich (1956) (amended by some personal observations) was used. Altogether, in the four collections, a total of 60 specimens was collected; thirty-five of these, which proved to be *Asterias vulgaris,* were used.

Dissection of specimens

Specimens were separately soaked in $MgCl_2$ solution (8% in distilled water) until flaccid. This process relaxes the animal and prevents autotomy during dissection. Two standard measurements commonly used in sea-star taxonomy, R and r, were recorded for each specimen. R is the length from the center of the disc along the aboral surface of the ray to the tip; r represents the distance from the center to the edge of the disc in an interradial angle. Each animal was then transferred to fresh sea water and dissected under a Bausch and Lomb Stereo-Zoom microscope in the following fashion. Two major cuts were made along each ray, one aborally through the carinal series of ossicles from the tip of the ray to the center of the disc, and another similar longitudinal cut orally through the center of the roof of the ambulacral groove. This procedure separated the animal into five pieces, each consisting of halves of two adjacent rays, joined together at the interradial angle. All extraneous tissue was cut away from each of these pieces, leaving five interradii, each containing two complete units of the reproductive system. Four interradii were then fixed in Bouin's fluid; the fifth was preserved in 70% ethanol.

Procedures for major collections

Specimens were allowed to stand in Bouin's fluid for periods of up to one month, which thoroughly decalcified them. The five interradii representing each animal were separated into two groups and processed as follows:

Group 1. Two entire interradii were dehydrated in ethanol, embedded in paraffin in a vacuum oven, and sectioned serially either longitudinally or transversely to the axis of the rachis of the gonad (see Observations) at 7 μ to 10μ. One set of sections from each specimen was routinely stained with Mallory's Phosphotungstic Acid Hematoxylin (PTAH) (Lillie, 1965) for general histologi-

cal observations and to reveal details of muscular and connective tissues. Selected regions of the second set of sections were stained with the following techniques: (a) Gomori's silver stain (Gomori, 1947)—to differentiate "reticular" collagen fibers in various connective tissue components of the reproductive system; (b) Aldehyde Fuchsin (Cameron and Steele, 1959) counterstained with Halmi's stain (1952)—to differentiate between elastic connective tissue and muscle and other connective tissue; and (c) Periodic Acid Schiff (PAS) (Lillie, 1965)—to demonstrate the distribution and abundance of the haemal fluid in the haemal sinus (see Observations). The presence of glycogen and other polysaccharide complexes is indicated by this technique.

Group 2. Gonads in the remaining interradii were used for general observations of shape and form of the gonad. The interradius which had been preserved in 70% ethanol was macerated in KOH solution (2% in distilled water) for periods of 2 to 20 minutes, depending on the toughness of the tissues of the ray, until the ossicles could be seen clearly. This preparation was preserved and used for observations to determine the point of suspension of the gonad.

Electron microscopy

In July, 1972, 6 specimens of *Asterias vulgaris* were collected on the leeward side of the breakwater at the Isles of Shoals. These animals consisted of 2 females and 4 males. The specimens were fixed under field conditions in the following way. The gonads of the 6 animals were removed after each specimen had been relaxed separately in $MgCl_2$ solution. Several blocks of tissue, 3–4 mm thick, taken from various parts of each gonad, were fixed at room temperature for 20–30 minutes in 3% paraformaldehyde-glutaraldehyde (Longo and Anderson, 1969) in sea water. The blocks were then diced into 1 mm cubes of tissue, and fixation was continued in a small refrigerator at 4° C. for 1–2 hours. The tissues were rinsed in 3 20-minute changes of seawater and post-fixed for 2 hours in 1% OsO_4 in seawater, followed by 4 10-minute seawater rinses. Dehydration of the tissues was carried out in ethanol (10 minutes each in 25%, 50%, 70%, 95%, 100% and 100% solutions). The tissues were then transferred to a 10-minute rinse in propylene oxide: 100% ethanol, 1:1, followed by 2 10-minute rinses in 100% propylene oxide, and embedded in Epon 812/Araldite 506 mixture (Mollenhauer, 1963). Silver-gray sections were cut on a Porter-Blum MT-2B ultramicrotome using glass knives and subsequently picked up on 0.25% formvar-coated 200 mesh grids. All sections were stained with both 2% uranyl acetate (30 minutes) and lead citrate (15 minutes) (Reynolds, 1963) and examined on a Philips EM 300 operated at 80 kV.

The results from all these procedures have been combined to provide a detailed account of the morphology and histology of the gonad.

Observations

General morphology of the gonad

In either sex of a normal five-rayed specimen of *Asterias vulgaris,* the reproductive system comprises 10 separate units, two in each ray. Each unit is attached

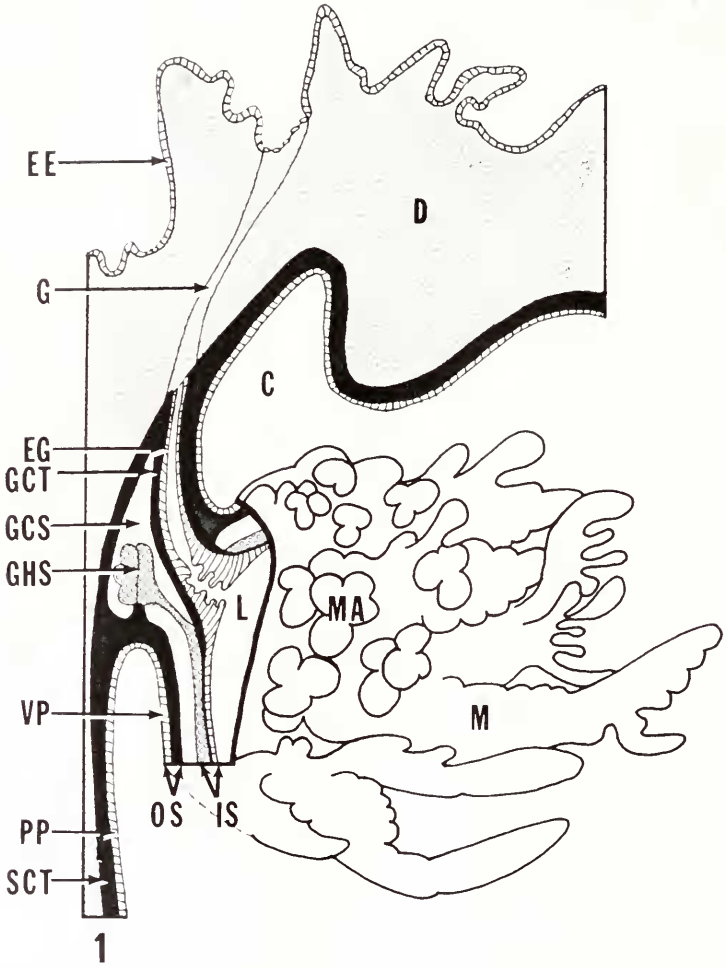


FIGURE 1. Diagrammatic representation of the inner lateral wall of the ray (on the left side) at the point of attachment of one unit of the reproductive system, showing the relationships of various layers of tissue which make up the gonad with those in the wall of the ray. Symbols used are: C, coelom; D, dermis; EE, external epithelium; EG, epithelium of the gonoduct; G, gonoduct; GCS, genital coelomic (perihaemal) sinus; GCT, gonoduct connective tissue; GHS, genital haemal strand; IS, inner sac; L, lumen of the gonad; M, major acinus; MA, minor acinus; OS, outer sac; PP, parietal peritoneum; SCT, subperitoneal connective tissue; VP, visceral peritoneum.

proximally near the disc on the inner face of the lateral wall of the ray, below the pyloric caecum, and the principal parts of the unit, the gonad, the gonoduct, and the genital haemal strand-genital coelomic (perihaemal) sinus complex, extend to other parts of the ray. The major features of a typical unit are shown diagrammatically in Figure 1. The gonad is large and obvious and projects freely into the coelomic cavity along the length of the ray; the gonoduct and genital

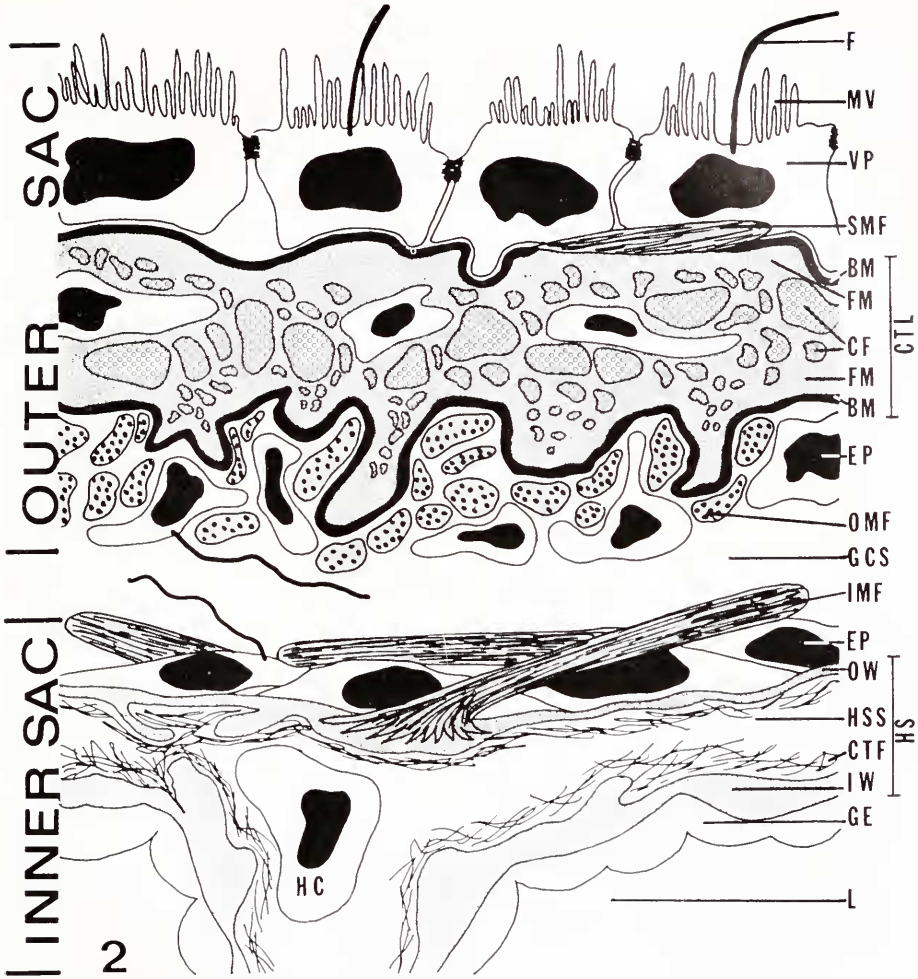
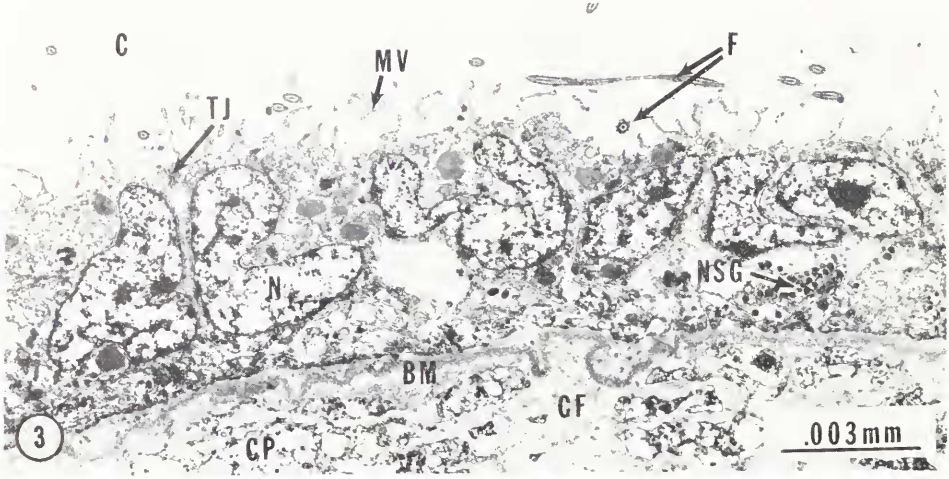


FIGURE 2. Diagrammatic representation of the typical arrangement and appearance of the tissues in the wall of the gonad. Symbols used are: BM, basement membrane; CF, collagen fibers; CTF, connective tissue fibers; CTL, connective tissue layer; EP, epithelium; F, flagellum; FM, fibrous matrix; GE, germinal epithelium; GCS, genital coelomic (perihaemal) sinus; HC, cell in the haemal sinus; HS, haemal sinus; HSS, haemal sinus space; IMF, inner longitudinal muscle fibers; IW, inner wall of haemal sinus; L, lumen of the gonad; MV, microvilli; OMF, outer circular muscle fibers; OW, outer wall of haemal sinus; SMF, subperitoneal muscle fibers; VP, visceral peritoneum.

haemal strand (and its associated coelom) are microscopic, the former penetrating the wall of the ray and opening aborally by several gonopores in the epithelium of the interradius, the latter connecting with the aboral haemal and coelomic rings in the disc.

In both sexes, the gonad is a single large bag consisting of a number of parts. A major piece, the rachis, forms the axis of the gonad; by this piece the gonad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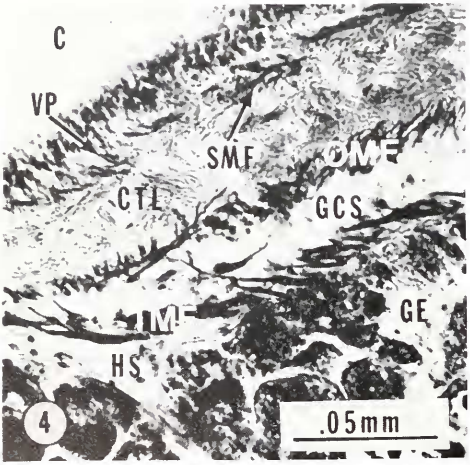
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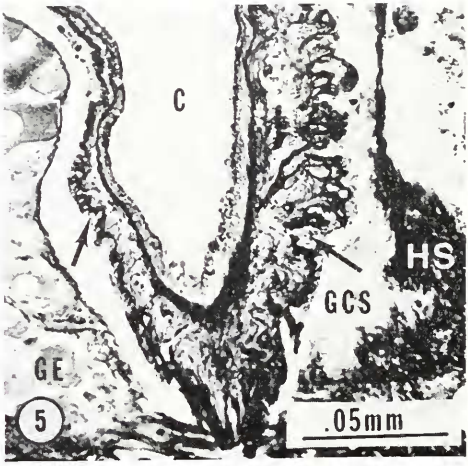
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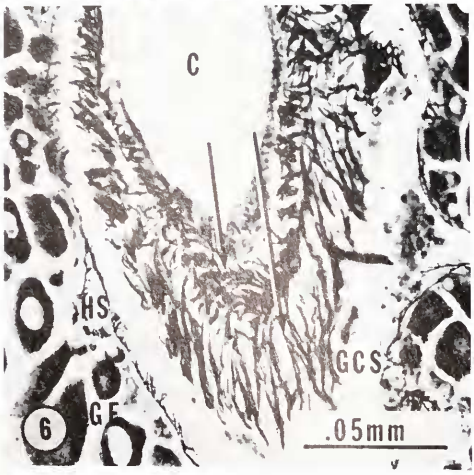
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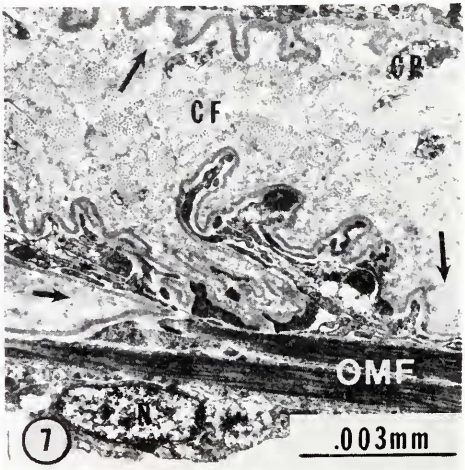
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is suspended from the body wall through a single stalk of connective tissue above the supra-marginal ossicles of the ray. Many projections extend from this axial piece into the coelom. A varying number (10–30) of elongate tubules, the major acini, project from the medial margin of the rachis toward the ambulacral plates. The remaining surface of the rachis, and often much of the major acini, are marked by short rounded protuberances, the minor acini.

Histology of the gonad

The wall of the gonad is composed of several layers of tissue arranged in two groups, the outer and inner sacs, details of which are shown in Figure 2. Although these layers are always found in the same order wherever they occur, their relative dimensions and general appearance differ markedly depending on the part of the gonad they form and on the degree of gametogenic activity occurring in the germinal epithelium at the time.

The *outer sac* consists of a single stratum of visceral peritoneal cells that rests on a layer of fibrous connective tissue to which muscle and epithelial cells are attached internally. The *inner sac* of the gonad is often very thin, complex, and difficult to interpret; full understanding of its structure necessitates the use of low magnification electron microscopy. It is composed of the haemal sinus and associated tissues. In this paper, "haemal sinus" refers to a genital branch of the aboral haemal ring found in association with the germinal epithelium of the gonad. The term is used collectively to include (1) the haemal sinus space, which may contain (2) granular PAS-positive haemal fluid and cells, enclosed by (3) two fibrous layers, called the outer and inner walls of the haemal sinus. The outer wall serves externally as a basement membrane for muscle and epithelial cells

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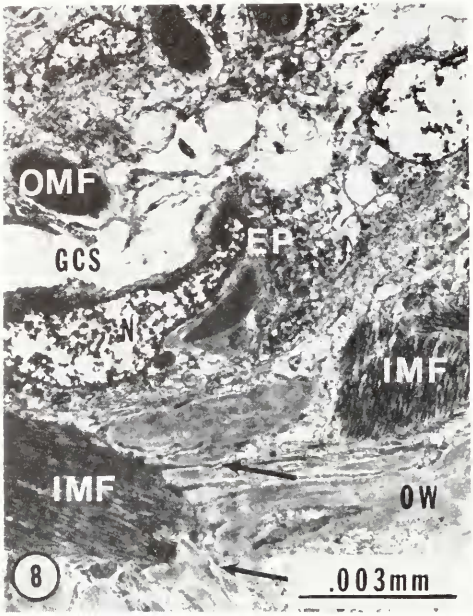
FIGURE 3. Electron micrograph of a section of a testis, showing the cells of the visceral peritoneum; Uranyl acetate and lead citrate. Symbols used are: BM, basement membrane; C, coelom, CF, collagen fibers; CP, cellular process; F, flagellum; MV, microvilli; N, nucleus; NSG, neurosecretory granules; TJ, tight junction.

FIGURE 4. Cross-sectional view of the wall of an ovary to show the general appearance of the tissues that compose it. Compare with Figures 2, 7, and 15. PTAH. Symbols used are: C, coelom; CTL, connective tissue layer; GE, germinal epithelium; GCS, genital coelomic (perihaemal) sinus; HS, haemal sinus; IMF, inner longitudinal muscle fibers; OMF, outer circular muscle fibers; SMF, subperitoneal muscle fibers; VP, visceral peritoneum.

FIGURE 5. A section of the wall of an ovary at the base of a minor acinus where the darkly staining borders of the outer connective tissue layer are obvious; muscle and epithelial cells lie within the folds of the inner border (arrows; lightly stained here); Aldehyde fuchsin, Halmi's stain. Symbols used are: C, coelom; GCS, genital coelomic (perihaemal) sinus; GE, germinal epithelium; HS, haemal sinus.

FIGURE 6. Section of the wall of an ovary at the base of a minor acinus, showing the distribution of the fibers of the outer connective tissue layer (arrows) and the connective tissue fibers of both walls of the haemal sinus near the germinal epithelium; Gomori's silver stain. Symbols used are: C, coelom; GCS, genital coelomic (perihaemal) sinus; GE, germinal epithelium; HS, haemal sinus.

FIGURE 7. Electron micrograph of a section of an ovary showing features of the connective tissue layer of the outer sac of the gonad. Notice, in particular, the wavy basement membranes of the visceral and genital-coelomic peritoneal layers; the arrows indicate the fine fibers of the matrix. Compare with Figure 4; Uranyl acetate and lead citrate. Symbols used are: CF, collagen fibers; CP, cellular processes; N, nucleus of an epithelial cell; OMF outer circular muscle fibers.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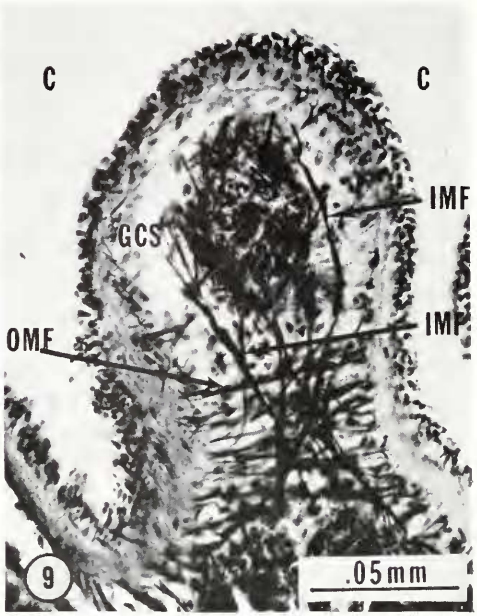
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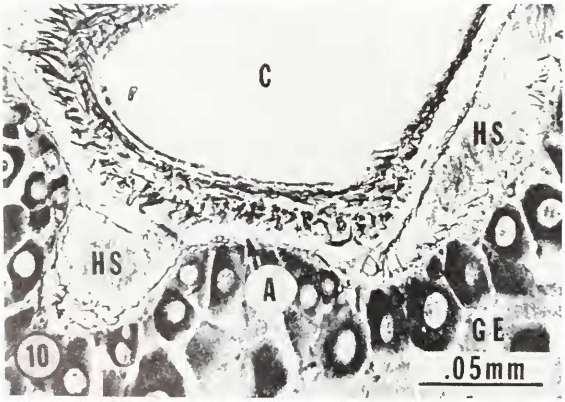
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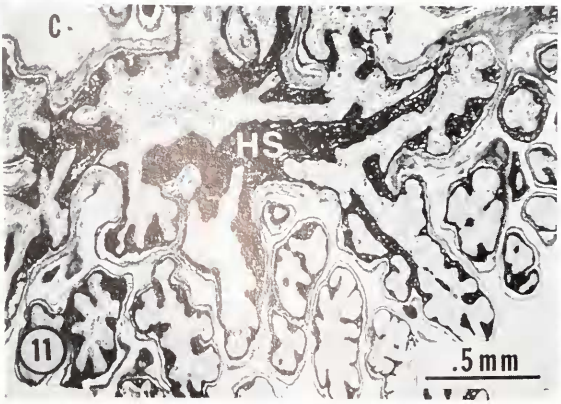
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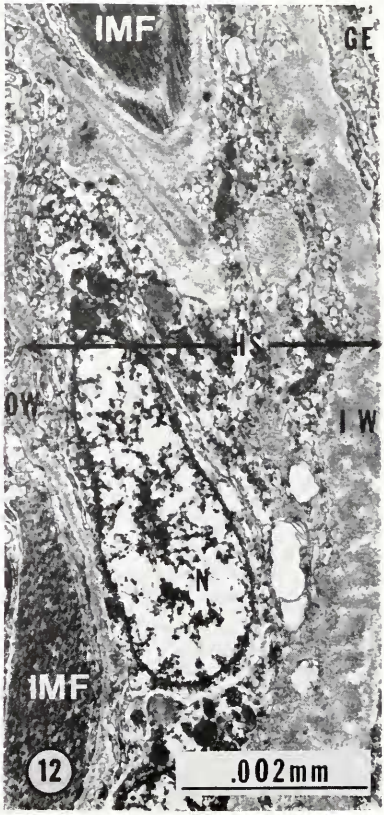
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found in the genital coelomic (perihaemal) sinus; the inner wall serves internally as a basement membrane for the germinal epithelium.

A distinct genital coelomic (perihaemal) sinus lies between the sacs which make up the wall of the gonad (Fig. 2, GCS). This sinus extends through the connective tissue stalk suspending the gonad and in the wall of the ray connects with a genital branch of the aboral coelomic (perihaemal) ring (Fig. 1, GHS). Such an attachment exists for each gonad, and thus all ten gonads are interconnected by way of this coelomic sinus. Both sacs are attached to the connective tissue of the gonoduct near its orifice within the gonad (Fig. 1). Elsewhere, although the two sacs may be in contact and nearly indistinguishable from each other at the limit of resolution of the light microscope, observations utilizing the electron microscope show clearly that they remain physically distinct and maintain their separate identities. In the living animal, a fluid is undoubtedly present in the genital coelomic (perihaemal) sinus, but no trace of it is evident in fixed, sectioned, and stained material. We now proceed to describe specific components of the wall of the gonad in greater detail.

The visceral peritoneal cells of the gonad are directly continuous with those of the parietal peritoneum (Figs. 1, 2, and 4, VP). The concept of these cells which is formed from observations using the light microscope is relatively simple and incomplete, because the cells are quite small and are usually not well preserved. The epithelial cells are basically cuboidal; however, their shape is quite variable and depending upon the degree of expansion of the gonad, the cells may be flat, cubical, or tall and columnar in appearance on various regions of the same gonad. In gonads that have completed gametogenesis, most cells are flat (squamous), while in spent gonads or those involved in gametogenesis, all three types of cells may be found. Observations with the electron microscope reveal additional details (Fig. 3). The cytoplasm of most cells contains few organelles, although a large

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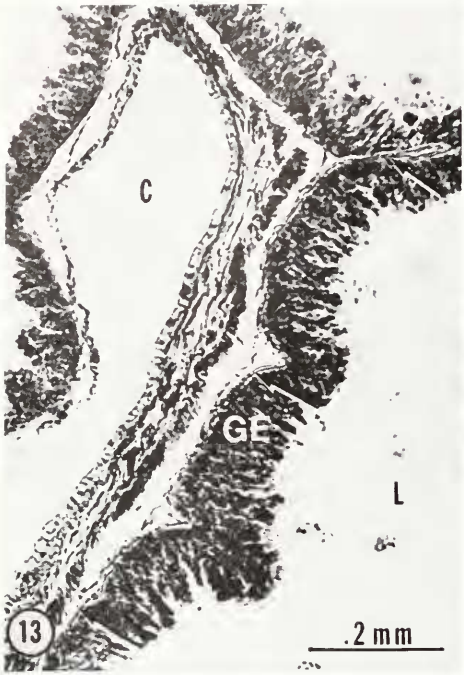
FIGURE 8. Electron micrograph of a section of an ovary, showing muscle fibers of the outer and inner sacs of the gonad. Notice the firm attachment of the inner longitudinal muscle fibers to the outer wall of the haemal sinus (arrows); Uranyl acetate and lead citrate. Symbols used are: EP, epithelium covering the outer wall of the haemal sinus; GCS, genital coelomic (perihaemal) sinus; IMF, inner longitudinal muscle fibers; N, nucleus of epithelial cell; OMF, outer circular muscle fibers; OW, outer wall of haemal sinus.

FIGURE 9. Longitudinal section of one minor acinus from an ovary, showing the orientation of the outer (OMF) and inner (IMF) muscle fibers; PTAH. Symbols used are: C, coelom; GCS, genital coelomic (perihaemal) sinus.

FIGURE 10. Section of an ovary, showing adjacent regions of the haemal sinus which appear either as a jumble of connective tissue fibers (A) or as an expanded space (HS); the connective tissue fibers are embedded on the luminal side of both the outer and inner walls of the sinus; Gomori's silver stain. Symbols used are: C, coelom; GE, germinal epithelium.

FIGURE 11. Several major acini sectioned longitudinally showing the general distribution of the haemal sinus and its PAS-positive contents; PAS. Symbols used are: C, coelom; HS, haemal sinus.

FIGURE 12. Electron micrograph of a section of an ovary, showing the haemal sinus and its components in a region where the walls of the sinus are close together. The haemal sinus space contains cells and not haemal fluid; Uranyl acetate and lead citrate. Symbols used are: GE, germinal epithelium; HS, haemal sinus; IMF, inner longitudinal muscle fibers; IW, inner wall of haemal sinus; N, nucleus of a cell in the haemal sinus; OW, outer wall of haemal sinus.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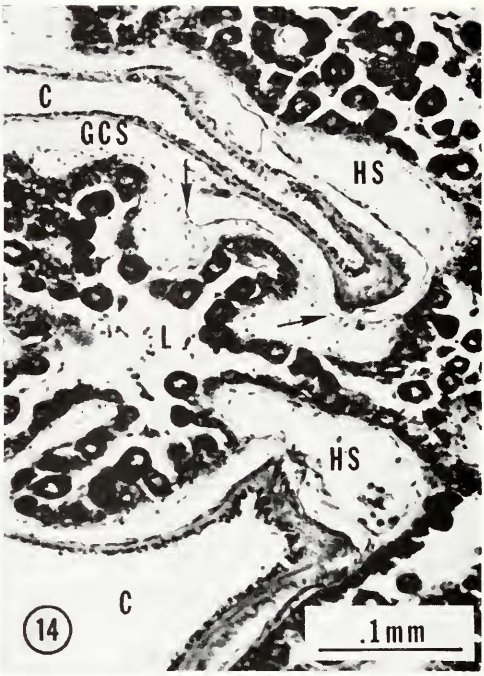
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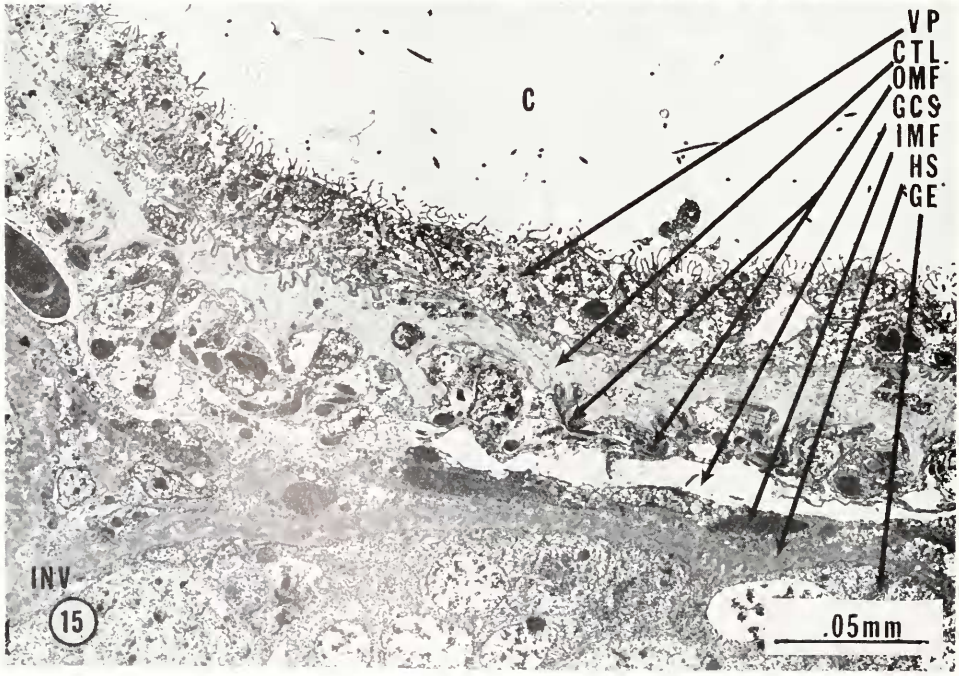
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nucleus, golgi apparatus, mitochondria, flagellar basal bodies, and granules are present. Microvilli surround the base of the single flagellum of each cell as a distinct circle and are also distributed randomly over the free surfaces of cells. The peritoneal cells are attached to each other by tight junctions, often through extremely attenuated processes. Membrane bound granules are found between and below the typical peritoneal cells (Fig. 3, NSG) and are similar to structures identified by Bruslé (1969) and Davis (1971) as nerve cell processes.

Fibers which stain yellow with Orange G and blue with PTAH but do not stain with silver are occasionally found in thick regions of the outer sac below the peritoneum surrounding the bases of the major and minor acini (Figs. 2 and 4, SMF). Such fibers may form a network at the base of the peritoneum; preliminary observations with the electron microscope show that they are muscle fibers.

The outer connective tissue layer (Figs. 2 and 4, CTL), is continuous with a similar layer located subperitoneally in the wall of the ray (Fig. 1, SCT). This continuity is evident across the stalk suspending the gonad, which is composed exclusively of tissues corresponding to those of the outer sac of the gonad (Fig. 1). In the animals observed, the maximum thickness of this layer is 30 μ to 35 μ; it is thickest along the wall of the rachis especially around the bases of the major acini and near the point of suspension of the gonad. In other places, as on the distal tip of the gonad or on rounded surfaces of the acini, it may be 2 μ or less in thickness. With the light microscope, the connective tissue layer appears to be condensed on both borders, and these regions stain dark blue with aldehyde fuchsin (Fig. 5). Sandwiched between these two condensed laminae is a thick zone of fibers which stain pink with PTAH (Fig. 4, CTL) and also stain with Gomori's silver technique (Fig. 6, arrows). This layer is quite elastic and resilient even in preserved material. Observations of this connective tissue with the electron microscope (Fig. 7) support and extend the interpretation just presented. The entire layer is composed of a matrix of fine fibers which is often interrupted or replaced by collagen fibers of various sizes running in all directions and by occasional groups of cells or cellular processes. The outer and inner margins of this layer, seen as condensed

FIGURE 13. Section of a testis showing the thin inner wall of the haemal sinus as seen by light microscopy (arrows) and the indistinct nature of the outer wall of the sinus; PTAH. Symbols used are: C, coelom; GE, germinal epithelium; L, lumen of the gonad.

FIGURE 14. Section of a minor acinus of an ovary showing the single cell layer covering the outer wall of the haemal sinus (arrows) (see Figure 8) and the invaginations projecting into the lumen of the gonad formed by the inner wall of the haemal sinus and the germinal epithelium; PTAH. Symbols used are: C, coelom; GCS, genital coelomic (perihaemal) sinus; HS, haemal sinus; L, lumen of the gonad.

FIGURE 15. Electron micrograph of the wall of an ovary taken at low magnification. Using this figure, compare the resolution of details of the various tissue components of the wall of the gonad with that seen in light micrographs of similar subjects, in particular, Figure 4; Uranyl acetate and lead citrate. Symbols used are: C, coelom; CTL, connective tissue layer; GE, germinal epithelium; GCS, genital coelomic (perihaemal) sinus; HS, haemal sinus; IMF, inner longitudinal muscle fibers; INV, invagination of the inner wall of the haemal sinus and the germinal epithelium; OMF, outer circular muscle fibers; VP, visceral peritoneum.

borders with the light microscope, form basement membranes for the visceral peritoneum and muscle and epithelial cells of the genital coelomic (perihaemal) sinus, respectively. These basement membranes are usually wavy in appearance as seen with both the light and electron microscopes (Figs. 5 and 7) and are composed of fine fibers similar to those in the matrix with which they are intimately associated. Complex, sometimes large cells and attenuated cellular processes are found among the fibers of the connective tissue layer; these are assumed to be associated with the formation of fibers.

The muscle fibers attached to the inner basement membrane of the outer sac of the gonad (Figs. 2, 4, 7 and 8, OMF) stain yellow with Orange G, blue with PTAH, and not at all with Gomori's silver stain. These fibers run circularly around the axes of the rachis and acini of the gonad (Fig. 9). Viewed in sections, they often lie close against the connective tissue layer, within folds formed by this layer (Figs. 2, 5 and 7).

Observations with the electron microscope indicate that a discontinuous epithelium lines the genital coelomic (perihaemal) sinus throughout the gonad (Figs. 2, 7, and 8). The cells of this epithelium occur among muscle fibers of the outer and inner sacs; they are often amoeboid and many are flagellated. Membrane bound granules associated with the epithelial cells of the genital coelomic (perihaemal) sinus are similar to those seen in several sea-stars by Davis (1971).

While all these tissues of the outer sac of the gonad may vary in thickness and shape, the tissues of the inner sac are even more variable; their appearance depends upon the activity of the germinal epithelium. Muscle fibers are often numerous in association with the outer wall of the haemal sinus only (Figs. 2, 4, and 8, IMF); they are intimately connected to this wall externally (Fig. 8, arrows). Such fibers are oriented longitudinally along the axes of the rachis and acini forming a basket of muscle fibers (Fig. 9).

Tissues associated with the haemal sinus of the gonad are similar to those of the genital haemal strand with which the sinus is continuous near the gonoduct (Fig. 1). Through the light microscope, the sinus appears either as a single layer or often in adjacent regions as two layers separated by the haemal sinus space. These layers are quite indistinct but correspond to the walls of the haemal sinus. Silver staining reveals distinct, thin, connective tissue fibers (clearly collagen fibers as seen with the electron microscope) running in random directions on the luminal side of both walls (Fig. 10). At certain times of the year (in this study, October and July, in particular) separate channels of the haemal sinus expand and become continuous; the sinus space is filled with PAS-positive haemal fluid evident in sectioned and stained material as a reddish-pink coagulate (Fig. 11, HS). Details of the haemal sinus are resolvable with the electron microscope. Only with the high resolution provided by this instrument can it be appreciated that both walls of the haemal sinus are present throughout the gonad; they are often wavy in appearance and may send projections into the lumen of the haemal sinus (Fig. 12). Each wall is composed of a fine fibrous material similar in appearance to the matrix portion of the connective tissue layer of the outer sac. Except in very unusual cases, only the thin inner wall of the haemal sinus is discernable with the light microscope (Fig. 13, arrows), although, when the sinus

is maximally expanded, the existence of the outer wall is suggested by the single layer of epithelial cells which covers it externally (Figs. 2 and 8, EP and 14, arrows). Both walls of the sinus may stain brightly with PAS and aldehyde fuchsin. The inner wall of the haemal sinus and its associated germinal epithelium often form deep folds that project into the lumen of the gonad (Figs. 2, 4, 10, 11, 13, 14 and 15). In sections these may appear triangular or they may have various shapes; similar invaginations were noticed by Mauzey (1966) in *Pisaster* and Chia (1968) in *Leptasterias*. The haemal sinus space generally contains granular fluid in which collagen and other fine fibers are often apparent; the orientation of these fibers may be random or may parallel the walls of the sinus. Cells with various shapes and contents occur throughout the sinus space (Figs. 12 and 15); some are amoeboid with long cytoplasmic processes and others are contracted and filled with granules.

In mature animals, the germinal epithelium forms a continuous lining of the lumen of the gonad; it connects with the epithelium of the gonoduct (Fig. 1, EG). The germinal epithelium is composed of follicular cells, oogonia, and oocytes in females and spermatogonia, spermatocytes, and maturing spermatozoa in males. Of all the tissues mentioned, seasonal changes are most dynamic in the germinal epithelium in connection with its yearly production of gametes. These changes have been described by a number of authors for several sea-stars and the essentially similar changes seen in *Asterias vulgaris* will not be discussed here.

Discussion

The observations just concluded provide a detailed description of the gonad of *Asterias vulgaris* in morphological and histological terms; morphologically the gonads of this species differ from those seen in most other sea-stars, histologically they appear to be quite similar to those in any asteroid. From the standpoint of microscopic anatomy it is fortunate that descriptive studies of various aspects of the histology of the gonads of asteroids are numerous. Collectively these provide a good outline which is applicable in general to almost any sea-star. As mentioned earlier, however, no studies are available which give a detailed description of the tissues that make up the wall of the gonad in both sexes of any species based on observations using both the light and electron microscopes. Many features of the general organization of various tissue layers of the gonad are obvious only with the light microscope, while details of the histology of the tissues are obvious only with low magnification electron microscopy. In the following section, the histology of the wall of the gonad of *Asterias vulgaris* will be compared with corresponding features of the gonads of other sea-stars which have been previously described.

The visceral peritoneum of the outer sac of the gonad has been described by some authors as a single layer of ciliated cuboidal cells (Field, *Asterias vulgaris*, 1892; Gemmill, *Solaster endeca*, 1912; and Chia, *Leptasterias hexactis* and *Henricia sanguinolenta*, 1968, 1970). In contrast, some observers state that it consists of ciliated flat cells (Ludwig and Hamann, 1899, and Davis, 1971). From the present study, it is apparent that this layer is basically composed of simple flagel-

lated cuboidal cells like those which make up the peritoneum elsewhere. Local pressures and stresses result in their being stretched thin over protuberances or laterally compressed in folds and angles. Preliminary observations on the ultrastructure of the peritoneal cells in the present study are in general agreement with those of Tangapregassom and Delavault (1967) and Davis (1971). Bruslé (1969) and Davis (1971) indicate that nerve cell processes containing granules that are possibly neurosecretory in nature are often seen below and between the peritoneal cells of the gonads of the sea-stars with which they worked. Granules were observed in the present study in a similar relationship with the visceral peritoneum, but the evidence is insufficient to confirm identification of such granules with nervous tissue in *Asterias vulgaris*.

The muscle fibers seen in association with the peritoneum of the gonad in *Asterias vulgaris* have never been described in other species. Their function in the gonads of this species is unknown, although they might cause local deformations of the peritoneum that direct movement of coelomic fluid over the surface of the gonad.

Hoffmann (1872), in his studies of *Asterias rubens*, was the first investigator to mention the connective tissue of the outer sac of the gonad, describing it as composed of delicate homogeneous connective tissue. More detail is presented by Gemmill (1914), who notes that in *Asterias rubens* the outer connective tissue layer may be subdivided into superficial and deeper sheets. Tangapregassom and Delavault (1967), Bruslé (1969), and Davis (1971) state that it is composed of collagen fibers in which there are occasional pockets of cells; other investigators mention that it exists but give no details. It is apparent from observations described earlier that this layer is extremely tough and elastic in *Asterias vulgaris*. Its borders serve as basement membranes, stain brightly with aldehyde fuchsin, are usually wavy in sections, and are very likely similar to the elastic tissue of vertebrates. The elastic basement membranes of this layer allow the gonad to expand while the abundant collagen fibers found between them maintain its basic shape.

The circular muscle fibers facing the genital coelomic (perihaemal) sinus on the inside of the outer connective tissue layer are similar to the circular fibers described by Hoffmann (1872) for *Asterias rubens* and by Gemmill (1912) in the ovary of *Solaster endeca*. Hayashi (1935), Chia (1968), Tangapregassom and Delavault (1967), Bruslé (1969), and Davis (1971), all recognize muscle fibers in a similar location in the gonads of the various species with which they worked, but according to Hayashi (1935), quoting from the observations of Ohshima (1925), this layer is not present in the testes of *Asterina batheri*. Contraction of these muscle fibers is probably important during the shedding of gametes.

The epithelial cells which line the genital coelomic (perihaemal) sinus of the gonad are very likely related to those in the wall of the ray which line the genital branch of the aboral coelomic (perihaemal) ring; they are flagellated and probably keep the fluid contents of the genital coelomic (perihaemal) sinus in motion.

Longitudinal muscle fibers were described by Gemmill (1912) on the inner sac of the gonad of *Solaster endeca*. Hayashi (1935), Chia (1968), Tangapre-

gassom and Delavault (1967), Bruslé (1969), and Davis (1971) also mention muscle fibers, similarly located, in the gonads of various species, without specifying their orientation. In *Asterias vulgaris*, as mentioned, longitudinal muscle fibers are attached only to the outer wall of the haemal sinus facing the genital coelomic (perihaemal) sinus. The function of these fibers remains unknown, although by their contraction they may exercise some control over the volume of fluid contained in the haemal sinus.

Although most previous investigators have mentioned the presence of tissues associated with the haemal sinus, none has given a detailed description of the components of the sinus. Taken together, the works of Gemmill (1914) and Davis (1971) give the most complete account. Gemmill indicates that a branch of the genital haemal ring connects with the inner sac of the gonad near the gonoduct and then branches irregularly over the surface of this sac; Davis describes the haemal sinus as a space filled with granular contents, cells, and collagen fibers, surrounded by two laminae. From the present study, it is clear that the sinus is an expansible bag with walls that are possibly elastic in character; the extreme variability in form and appearance of the haemal sinus is closely associated with changes in the condition of the germinal epithelium. In gonads which have completed gametogenesis, the sinus is nearly empty, its walls are pressed together, and the haemal sinus space is occluded. Alternatively, in gonads which are either involved in gametogenesis or have spawned recently, the haemal sinus may be filled with fluid and cells and its walls are widely separated. Work by Mauzey (1966) and Chia (1968) has shown that haemal fluid is strongly PAS-positive. Mauzey (1966) finds that the haemal fluid in *Pisaster ochraceus* does not contain glycogen since haemal fluid is PAS-positive before and after salivary amylase treatment. Chia concludes that this fluid may store and supply nutrients to the developing germinal cells, citing as evidence its location and the time during the reproductive cycle when it becomes conspicuous. In the present study, haemal fluid was present in greater volume in females than in males; it was found while gametes were maturing and was very abundant after gametes had been shed. No evidence is available on the function of this fluid in *Asterias vulgaris*.

Through the aboral haemal ring and its genital branches, the haemal sinus of each gonad is connected with those found in all 9 other gonads and also indirectly to other organ systems in the body: the pyloric caeca, axial organ, radial nerves of the rays, etc. Such a system of channels between the reproductive organs and other organ systems is no doubt important in transmitting fluids and other materials to and from the gonads, and also in coordinating the activities of all 10 gonads during the annual reproductive cycle.

From every aspect, the studies reported here reveal the limited amount of detailed information upon which our understanding of structure and function in the gonads of asteroids is based. It is thus obvious that additional studies, utilizing as many and varied species as possible, are highly desirable.

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Summary

The results reported here provide a detailed account of the general morphology and histology of the male and female gonads of the sea-star *Asterias vulgaris*. The reproductive system of this sea-star (normal five-rayed specimens) consists of 10 separate units, each located proximally on the lateral wall of the ray, one on either side of the ray. Each unit is composed of a gonad, a gonoduct, and genital branches of the aboral haemal and coelomic rings. The gonad is a single bag-like structure with several protrusions (termed major and minor acini) extending from its surface. Its wall is composed of two sacs, one inside the other, separated by the genital coelomic (perihaemal) sinus. The outer sac consists of visceral peritoneum, an elastic-collagenous connective tissue layer, and many epithelial cells and circular muscle fibers. The inner sac comprises epithelial cells and longitudinal muscle fibers, the haemal sinus and contents, and germinal epithelium. The haemal sinus includes the haemal sinus space, filled with granular haemal fluid, cells, and collagen and other fine fibers enclosed by two fibrous laminae. Significant modifications in the form of the gonad and in the condition and relationships of the tissues which compose it occur during the annual reproductive cycle. Both sacs are stretched during growth of the gonad, the outer layers becoming attenuated and the inner layers being pressed against the outer, often obliterating the genital coelomic (perihaemal) sinus. The inner group of tissues is often extensively folded, pushing ridges formed from the inner wall of the haemal sinus and germinal epithelium into the lumen of the gonad.

It is pointed out that previous studies on the gonads of asteroids have been relatively few, with no study for any species dealing comprehensively with morphological and histological details of the gonad based on both light and electron microscopy. Comparison of the results of the present study with observations of previous investigators indicates that although significant differences occur (especially in morphological terms), the general features of the histology of the wall of the gonad of many sea-stars are similar. In order to broaden our base for comparative studies, and to pursue significant problems, morphological, histological, histochemical, and ultrastructural investigations should be extended to as many asteroid species as possible.

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